Immuno-PCR (IPCR) technology for immunogenicity:

a comparative study between ECL and Immuno-PCR

EIP meeting Lisbon 2015

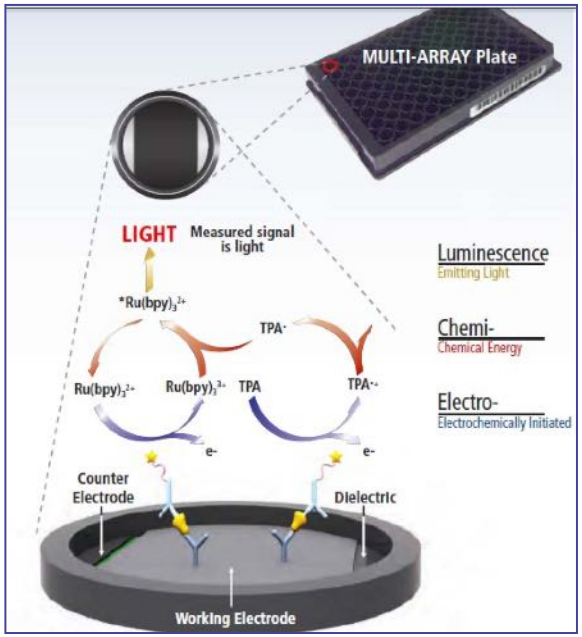
L. Vermet with special thanks to Mark Spengler from Chimera Biotec®

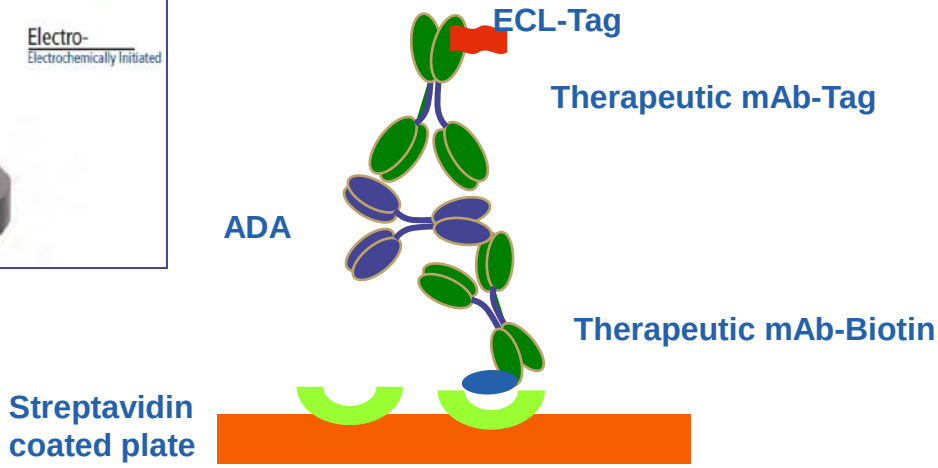
SANOFI Research & Development

Overview

- Electrochemiluminescence (ECL) ADA validated method with acid dissociation step
  - **Principle and overview**
  - **Summary of the method characteristics**
- Immuno-PCR
  - **Litterature and principle**
  - **Results of qualification items**
- Conclusions with a summary of Pro's and Con's of each technology
- Next steps

Electrochemiluminescence (ECL): Sector imager from Meso Scale Discovery®



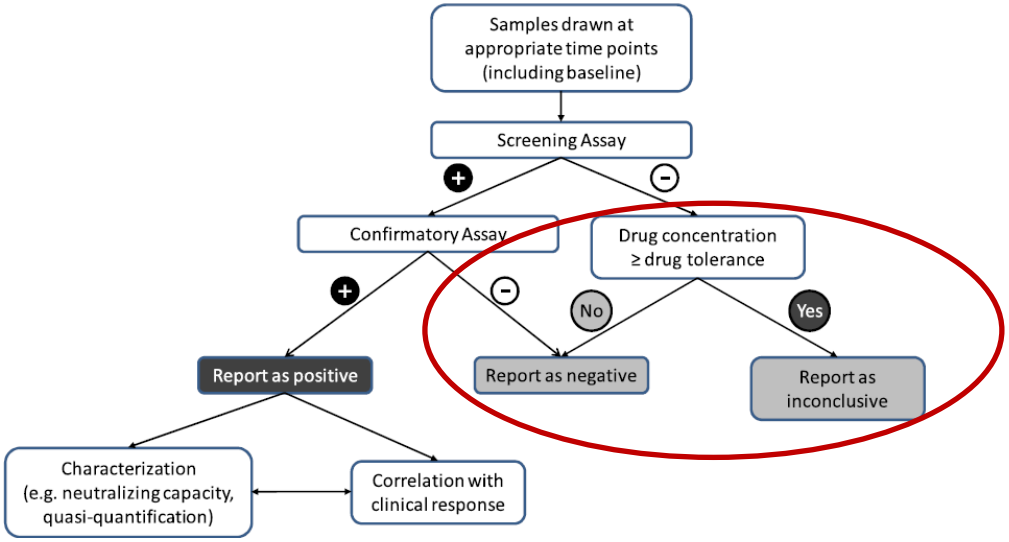


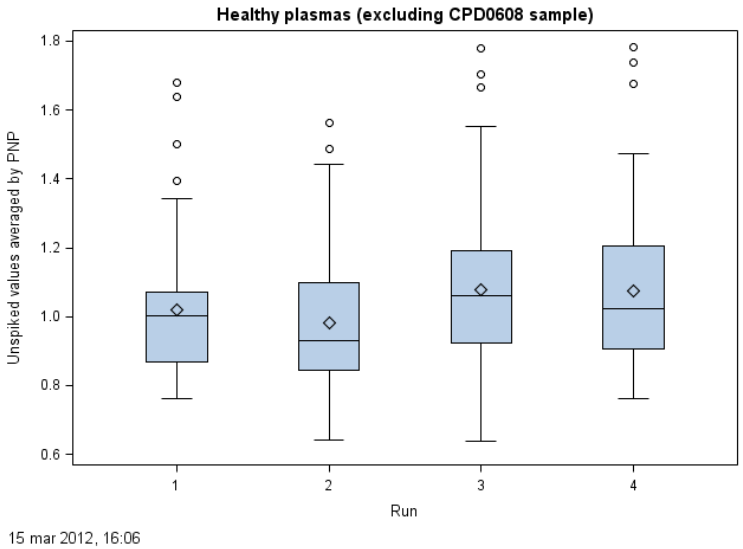
- MRD*: 1/30
- Acid dissociation step

Bridging format for qualitative detection of a Therapeutic mAb (SAR)

* Minimum Required Dilution

Full assay validation study of a Bridging format for qualitative detection of ADA in human plasma (1)





- Cut-point
  - **Normalization screening cut-point factor:**
    - normality assumption rejected,
    - non-parametric method based on the empirical 95th percentile: Ncut-point level at 1.55.
  - **Specificity cut-point:**
    - normality assumption rejected for the percent signal inhibition data,
    - non-parametric method based on the empirical 99.9th: confirmatory cut-point of 37.22%.

Kloks, C., et al., A fit-for-purpose strategy for the risk-based immunogenicity testing of biotherapeutics: a EIP, J. Immunol. Methods (2015),

Full assay validation study of a Bridging format for qualitative detection of ADA in human plasma (2)

Precision <20% on ADA PC levels

(Negative, LOW, MID and HIGH PC)

Long term, short term and freeze/thaw cycles stability validated

Sensitivity of the method: 100 ng/mL

Free drug tolerance (FDT)

- **20 µg/mL of Thera mAb on LOW PC (100 ng/mL)**
- **to be improved to support clinical studies**

No matrix variability

No hemolyse effect

Robust assay

Immuno-PCR Litterature: applications in the Bioanalysis area

The story of Immuno-PCR (iPCR) starts in 1992 with Sano

Sano, T., Smith, C.L., Cantor, C.R., 1992. Immuno-PCR: very sensitive antigen detection by means of specific antibody-DNA conjugates. Science 258, 120–122.

Immunodetection was combined with real-time PCR and used for quantification of vascular endothelial growth factor (Sims et al., 2000)

Different strategies applied for linking antibodies with DNA templates

- **streptavidin bridge combined with biotinylated antibody and biotinylated DNA template,**
- **chemically conjugated antibody-DNA complexes**

Lind and Kubista, 2005; Niemeyer et al., 2007

- Drug quantification in biological fluids
  - TK, PK methods

Pharmacokinetics of natural mistletoe lectins after subcutaneous injection. Hubet R Eur J Clin Pharmacol. 2010 Sep;66(9):889-97

- Immunogenicity
  - Screening and confirmatory assay
  - Nab testing

Immuno-PCR assays for immunogenicity testing. Spengler M Biochem Biophys Res Commun. 2009 Sep 18;387(2):278-82 .

- BM quantification
  - Safety BM
  - Clinical BM
  - Companion diagnostic

Potuckova L Journal of Immunological Methods. 371 (2010) 38-47.

Major Fields of Application

chimera biotec
ultra sensitive immunoassays

Special Pharmacokinetics

Case Studies:
1 Toxin derivatives / low dowsed drugs: Tarcha et al. AAPS NBC 2014
2 Peptide drug: Rat et al. AAPS NBC 2014
3 Biomarker analogous fusion-protein drug: Goyal et al. AAPS NBC 2012

Biomarker

Case Studies:
1 Human GM-CSF: Spengler et al. AAPS NBC 2013
2 Human INFg: Ancian et al. AAPS NBC 2012
3 Mucosal Vaccination: Fleury et al. 2012 (AAPS Innovation in Biotechnology Award)

Microsampling

Case Studies:
1 pTAU in mouse CSF: Smeraglia et al. EBF 2014
2 IL-2 & IL-6 for serial LMS: Pieper et al. EBF 2014

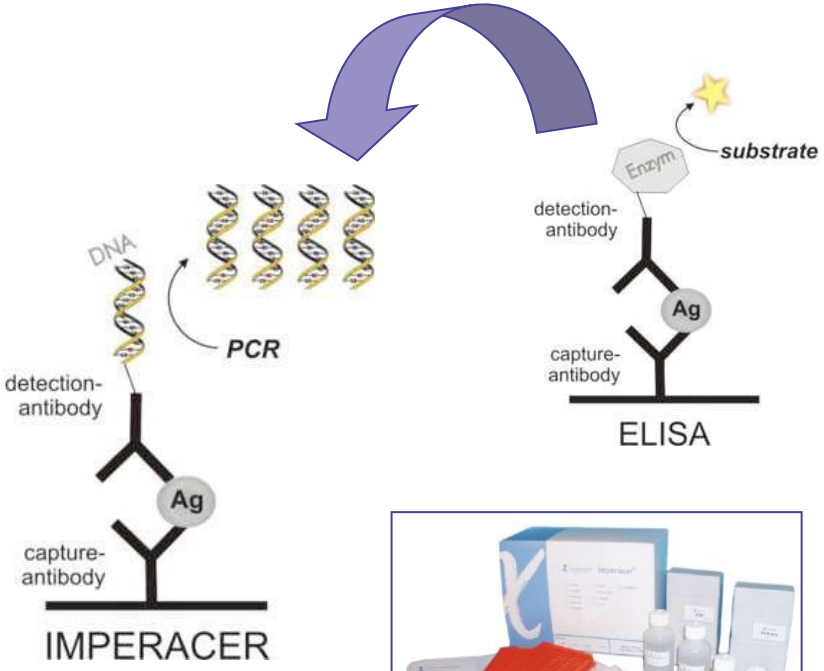
Immunogenicity

Case Studies:
1 From ELISA to IPCR: Goyal et al. EBF 2011
2 Tech comparison, MSD vs. Imperacer: Cortez et al. AAPS NBC 2014

Chimera Biotec®: company overview

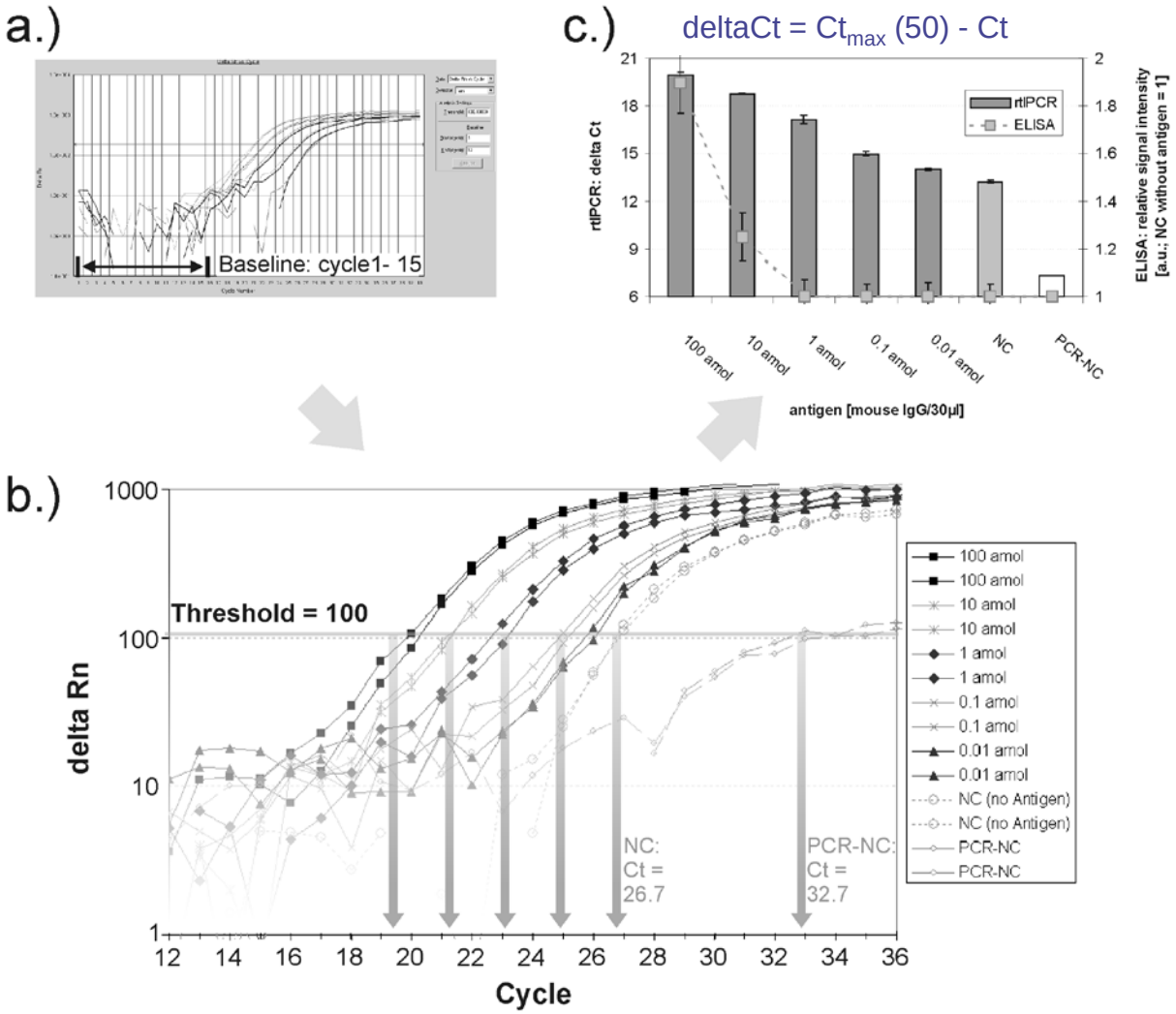
- **Founded in 2000**
- **Laboratory in Dortmund – Germany**
- **Marketing Imperacer® instrument since 2004**
- **Focus: "Ultra sensitive Immunoassays"**
- **Bioanalytical CRO Services**

Immuno-PCR principle: Imperacer® Workstation



- Antibody-DNA Conjugate replaces Antibody-enzyme Conjugate
- Processing & Read-out by real-time quantitative PCR (qPCR)
- Possibility of high dilution of sample maintaining high sensitivity (potential reduction of matrix effect and drug interference, low volume of sample)

Imperacer® Instrument Response: *DeltaCT*



a.) baseline calibration

b.) set threshold

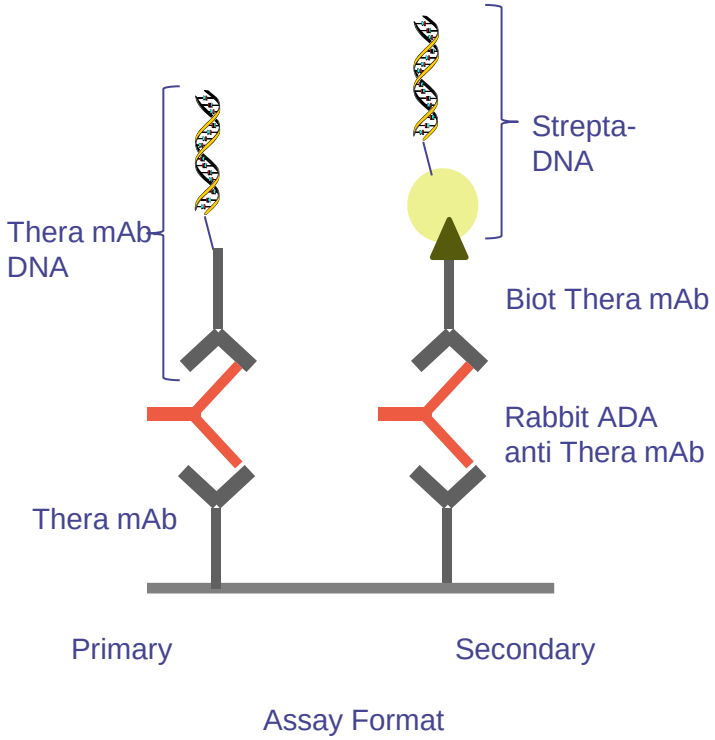
c.)calculate DeltaCt

(Ct$_{max}$ =50 cycles per each run)

Imperacer®

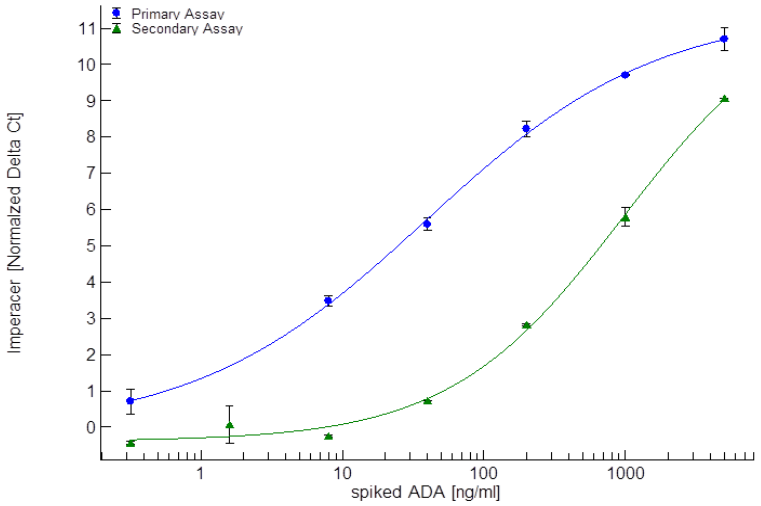
Anti-Drug-Antibody (ADA) Detection Method Development for human plasma

- Assay Setup and Method Optimization
  - **Acid dissociation was used in ECL and Imperacer® procedure**
  - **Same Antibodies used between MSD and Immuno-PCR**
  - **Comparison Primary versus Secondary Assay format in human plasma**



Primary/Secondary Assay in human plasma

"Reaching the limits of sensitivity"



4PL fit: the detection range was found to cover at least 4 orders of magnitude

- Direct DNA labeling (primary assay) format leads to a significantly better detection limit, however the secondary format demonstrated better performance under acid dissociation conditions
- => Selection of the secondary assay format for the other items of the study

Primary Assay:

Rabbit anti SAR [ng/ml] nominal	delta Ct average	delta Ct [NC=0] normalized	calculated concentration [ng/ml]	calculated concentration % nominal	calculated concentration %RE
5000	24.435	10.7	5073.301	101.47	1.47
1000	23.435	9.7	930.567	93.06	6.94
200	21.955	8.22	222.634	111.32	11.32
40	19.325	5.59	36.224	90.56	9.44
8	17.21	3.475	8.545	106.81	6.81
1.6	12.815	-0.92	Outlier rejected automatically		
0.32	14.445	0.71	0.307	95.83	4.17
NC	13.735	0	0.006		

A sensitivity of approx. **320 pg/mL** was detected in buffer and **human matrix** (without optimization for optimal detection limit)

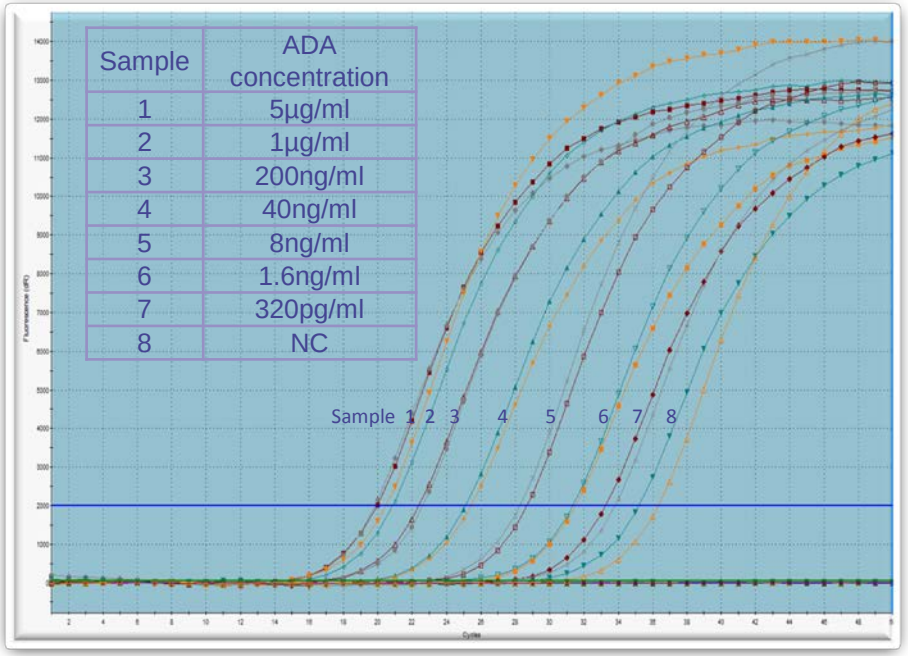
Secondary Assay:

Rabbit anti SAR [ng/ml] nominal	delta Ct average	delta Ct [NC=0] normalized	calculated concentration [ng/ml]	calculated concentration % nominal	calculated concentration %RE
5000	24.425	9.07	5074.970	101.50	1.50
1000	21.145	5.79	961.417	96.14	3.86
200	18.175	2.82	218.976	109.49	9.49
40	16.08	0.725	36.680	91.70	8.30
8	15.105	-0.25	1.735	21.69	78.31
1.6	15.425	0.07	9.679	604.95	504.95
0.32	14.92	-0.435	ND	ND	ND
NC	15.355	0	7.601		

Assay steps for Imperacer ® ADA method

ImmunoPCR Protocol

1. Imperacer® microplate modules (8-well strips) were incubated overnight at 4C with 5 µg/mL SAR antibody in Chimera's coating buffer (30 µl/well).
2. Dilution series of the ADA standard in Buffer/matrix
3. Samples and standard curve were diluted 1+9 in acetic acid (300mM) for 1h
4. Microplate wells were washed and blocked against unspecific interaction
5. Samples were mixed 1+1 with a 400ng/mL solution of Therapeutic mAb-biotin as detector in neutralizing assay buffer and incubated for 1h at room temperature on capture coated wells.
6. After a washing step a dilution of the detection conjugate DNA was incubated for 30min at room temperature in the wells.
7. Subsequent to a final washing step, PCR mastermix was added. Wells were sealed and analyzed in the Imperacer® reader (real time cycler, part of Imperacer® workstation).



Imperacer® detection ADA in spiked buffer (SDB6000). Real-time data read-out for the Imperacer® experiment; the experiment was carried out in a 2-fold determination. Ct values were determined for an automatic baseline correction, threshold was set to 2000.

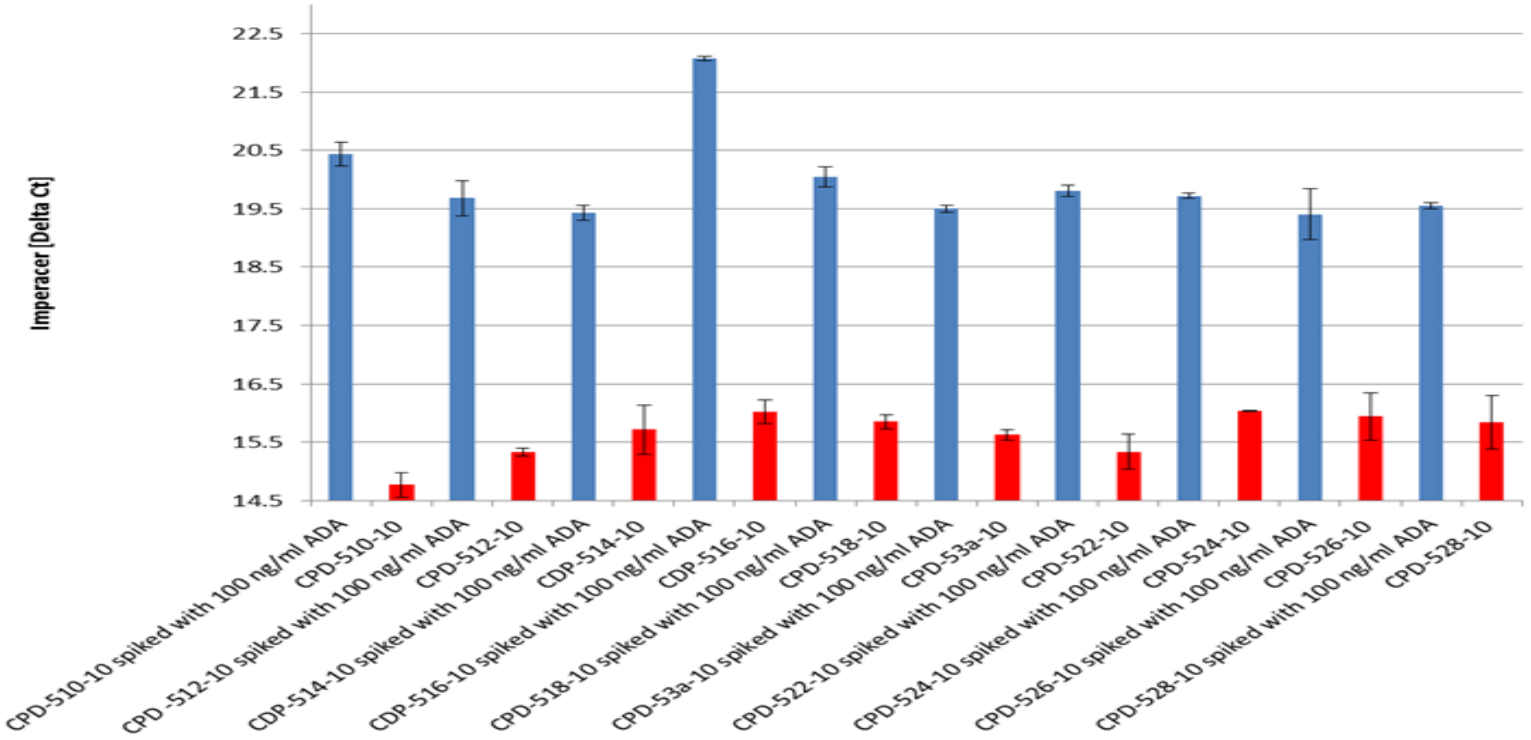
Minimum required dilution: 1:20

Pre-evaluation of Intra assay precision

Sample	ADA concentration in matrix	Ct (dRn)		Delta Ct (dRn)		average delta Ct	Intra-assay standard deviation	Standard deviation in % average (CV%)
1	5µg/ml	19.97	19.86	30.03	30.14	30.085	0.08	0.259
2	1µg/ml	20.89	20.52	29.11	29.48	29.295	0.26	0.893
3	200ng/ml	22.44	22.66	27.56	27.34	27.45	0.16	0.567
4	40ng/ml	25.13	25.46	24.87	24.54	24.705	0.23	0.233
5	8ng/ml	28.71	28.31	21.29	21.69	21.49	0.28	0.283
6	1.6ng/ml	31.38	31.55	18.62	18.45	18.535	0.12	0.120
7	320pg/ml	33.29	33.82	16.71	16.18	16.445	0.37	0.375
8	NC	35.23	36.37	14.77	13.63	14.2	0.81	0.806

- CV% below 1% on duplicate for all standard levels

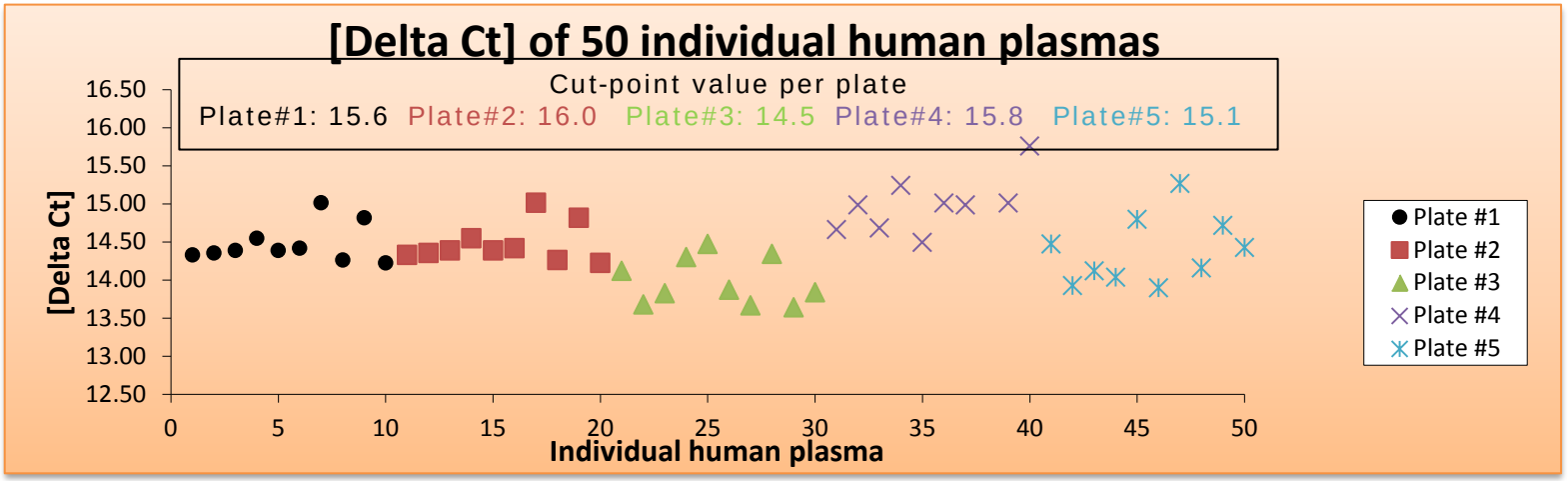
Matrix effect



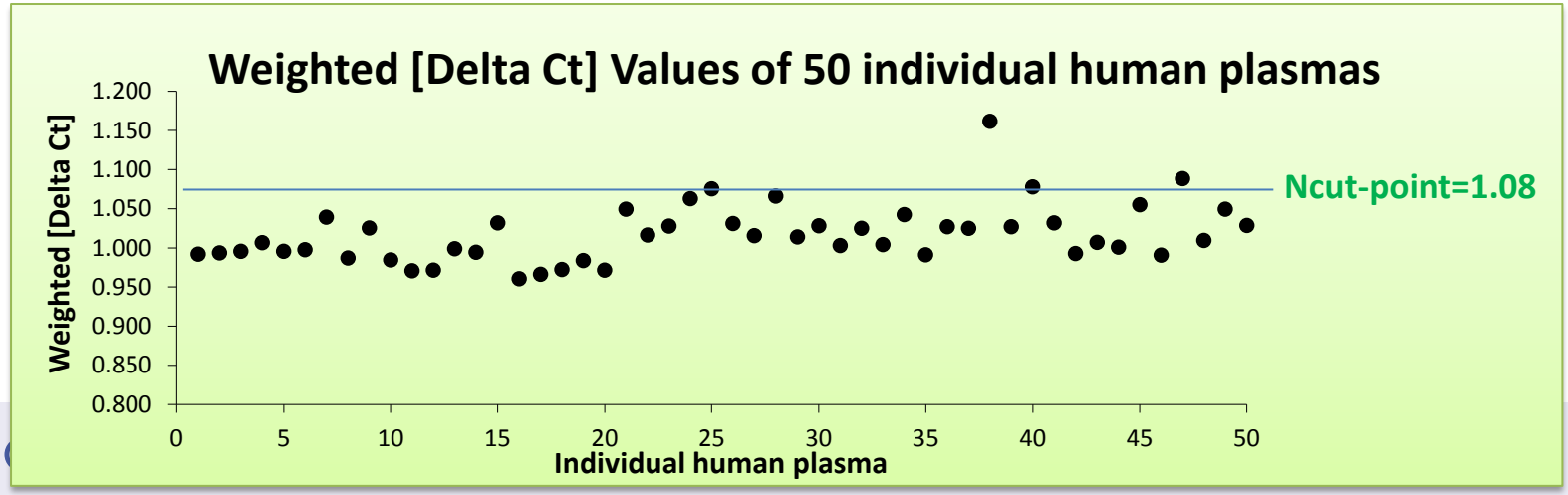
- 10 individual plasma samples were measured spiked with 100ng/ml ADA and non-spiked in absence of free Thera mAb.

  => No matrix effect

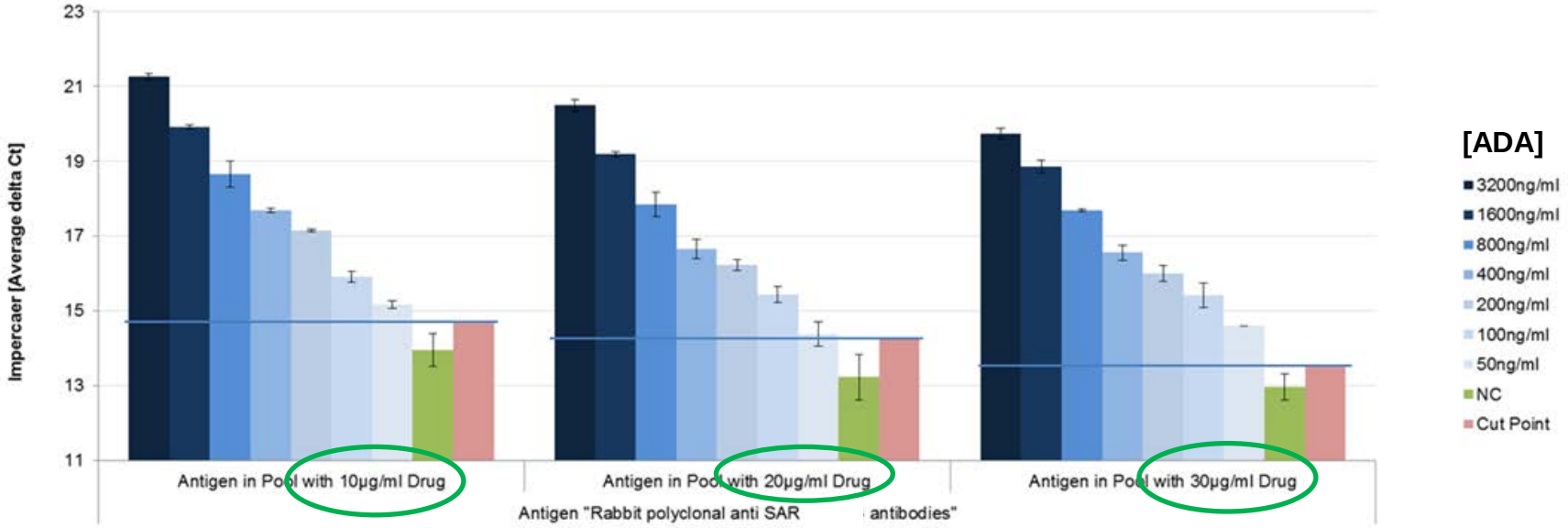
Screening Cut point evaluation on 50 plasma samples of healthy subjects



- The Cut Point calculation was performed for each Plate:
  - **Weighted Values = Delta Ct (Individual)/Delta Ct (NC)**
  - **N Cut-Point = Average Weighted Values + 1.645x St.Dev. (Weighted Values)**
  - **For routine analysis: Cut-Point of the plate= Delta Ct (NC) x N Cut-Point**



Free Drug Tolerance



- Discrimination between plasma pool spiked with 50ng/ml and non-spiked pool is possible even in presence of 30µg/ml free drug.

- The drug tolerance was improved to **30 µg/mL** of therapeutic mAb at concentration of ADA of **50 ng/mL** corresponding to a "therapeutic mAb /ADA" molar ratio of **600**.

Comparative conclusions on the ADA assays

	Validated ECL (MSD®)	Imperacer®
Sensitivity (ng/mL)	100	**0.320**
N Cut-point factor (Screening)	1.55	1.08
Matrix volume/run (µl)	<10	<10
MRD	1:30	1:20
Precision	<20%	<1% (Pre-evaluation on intra)
Matrix effect	no	no
Acid treatment	yes	yes
Free Drug Tolerance	Therapeutic mAb/ADA molar ratio: 200 (100ng/mL of ADA in presence of 20µg/mL of Drug)	Therapeutic mAb/ADA molar ratio: **600** (50ng/mL of ADA in presence of 30µg/mL of Drug)

- General method development of a bridging Imperacer® assay for anti-Drug antibodies detection is achievable.
- The Imperacer® technology is an interesting approach for ADA-testing.
- The relative improvement of sensitivity in presence of high amount of Drug indicates strong capabilities and potential for this technology.

Other comparative items: ECL vs Imperacer ® (a user point of view)

	Validated ECL (MSD®)	Imperacer®
Productivity Number of samples per day for 1 equipment and 1 analyst	30-60 samples/day	30-60 samples/day
Reagent cost	3	2
Equipment cost investment in equipment, software and training	40 K euros	100 K euros
Manpower cost	1 FTE / day	1 FTE / day
	1 - 3 runs /day	1 - 3 runs /day
LIMS interface	NO	NO
Open system / customization	4	4
availability of commercial kits	5	1
IQ -OQ availability	5	5
Availability in CRO	4	1
Multiplex	YES	NO

Score
1 = very poor
5 = excellent

Erenna™(Singulex)/Imperacer®(Chimera)/Simoa™(Quanterix)

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Emerging Technologies to Increase Ligand Binding Assay Sensitivity

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Abstract. Ligand binding assays (LBAs) have been the method of choice for protein analyte measurements for more than four decades. Over the years, LBA methods have improved in sensitivity and achieved larger dynamic ranges by using alternative detection systems and new technologies. As a consequence, the landscape and application of immunoassay platforms has changed dramatically. The introduction of bead-based methods, coupled with single molecule detection standardization and the ability to amplify assay signals, has improved the sensitivity of many immunoassays, in some cases by several logs of magnitude. Three promising immunoassay platforms are described in this article: Single Molecule Counting (SMC™) from Singulex Inc, Single Molecule Arrays (Simoa™) from Quanterix Corporation, and Immuno-PCR (Imperacer®) from Chimera Biotec GmbH. These platforms have the potential to significantly improve immunoassay sensitivity and thereby address the bioanalytical needs and challenges faced during biopharmaceutical drug development.

KEY WORDS: immunoassays; Immuno-PCR (Chimera Biotec GmbH); ligand binding assay (LBA); sensitivity; Single Molecule Array (Quanterix Corporation); Single Molecule Counting (Singulex Inc).

Next steps

- Complete the study with testing clinical study samples (Healthy and patient)
- Assessment of Imperacer® will continue on other biologics projects since the FDT remains problematic, in this context it can be a good alternative to other technological plateform.
- In addition to applications for immunogenicity we can also expect that this technology is of high utility for biomarker detection and pharmacokinetic purposes, for which we need to target a very high sensitivity below the pg/mL range in some cases.

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Back-up slides

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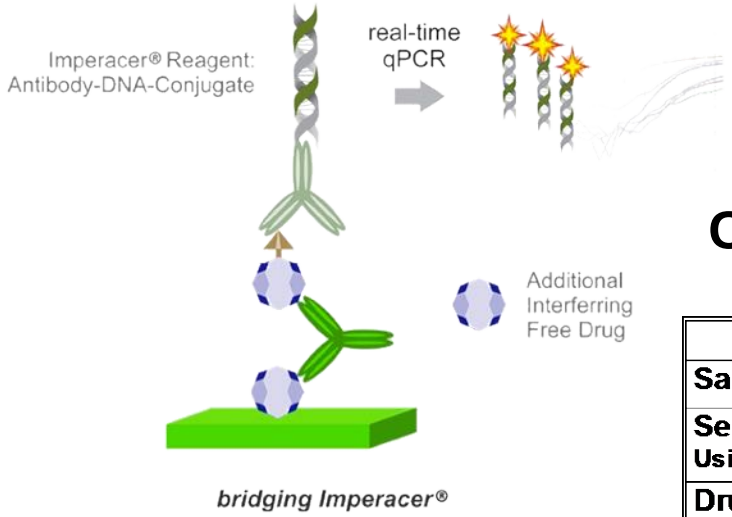
ADA Screening Assay

chimera biotec
ultra sensitive immunoassays



Comparison of Platforms

	ELI SA	MSD	I MPERACER®
Sample Volume	100 µl	100 µl	30 µl
Sensitivity Using mAB	70 ng/ml	20 ng/ml	40 pg/ml
Drug Tolerance (mAB : Drug)	1 : 1	1 : 40	1: 1000
Sample Pre-Treatment (Acid Dissociation)	Increased Background	Increased Background	NA
Matrix Interference	+++	+	NA

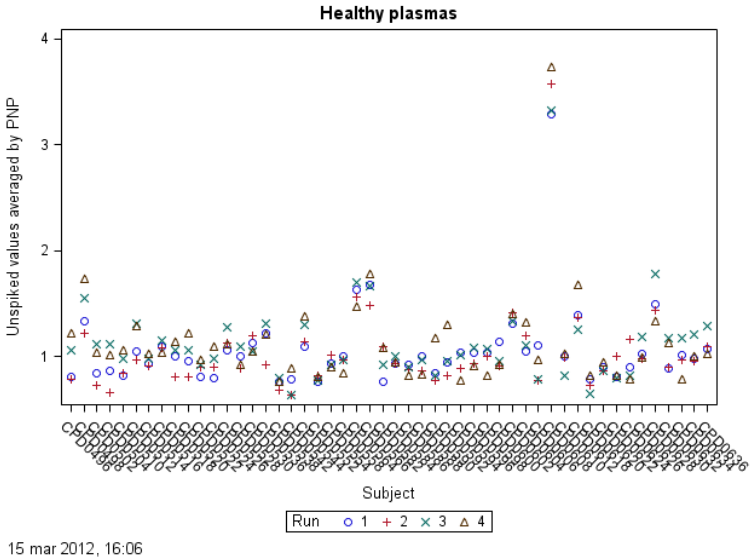
⇨ **Sponsor in-house ELISA & ECL assays compared to Imperacer®**

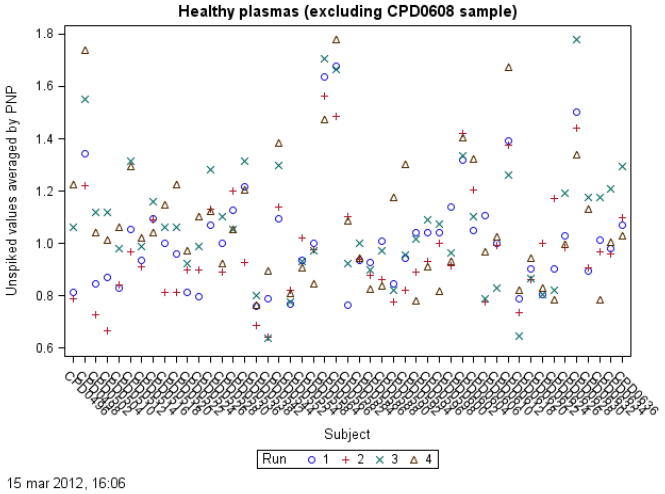
⇨ **Same Antibodies & Reagents used**

⇨ **No sample pre-treatment was used in Imperacer® procedure**

Please refer to: Imperacer® Presentations at the EBF open Conference, 16-18th Nov. 2011, Barcelona
Goyal et al. (Biogen IDEC, Cambridge, USA)

Cut point evaluation of MSD Bridging format





- Cut-point
  - **Normalization screening cut-point factor: normality assumption rejected, the non-parametric method based on the empirical 95th percentile was chosen giving an Ncut-point level at 1.55.**
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